

Mixed infections of cucumber fruits caused by diverse fungal pathogens under greenhouse conditions

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Abstract. Cucumber is among the most widely cultivated crops in greenhouses conditions in Kyrgyzstan. In recent years, parthenocarpic heterotic hybrids have become common in greenhouse production. However, the resistance or susceptibility of these hybrid varieties to fungal and bacterial diseases remains largely unexplored. This is a significant concern, as greenhouses create optimal conditions for pathogens due to elevated humidity and restricted airflow. Accurate and timely diagnosis of cucumber diseases is crucial for effective crop protection. Nonetheless, in Kyrgyzstan, the pathogens affecting cucumbers grown both in open fields and greenhouses have yet to be examined using specialised phytopathological methods to determine their unique morphophysiological and pathogenic characteristics. This study aimed to isolate and identify the main fungal pathogens affecting greenhouse-grown Björn F1 cucumbers and to evaluate their pathogenicity. Cucumber fruits displaying disease symptoms were collected for analysis. Both modern and classical phytopathological and microbiological techniques were employed to identify the pathogens, while their cultural and morphological traits were examined using light microscopy. The study identified fungal diseases in greenhouse-grown cucumbers caused by pathogens from the genera *Fusarium*, *Botrytis*, *Alternaria*, and *Cladosporium*. Pathogenicity tests were performed both *in vitro* and *in vivo*. The relative prevalence of each genus was as follows: *Fusarium* spp. (25%), *Botrytis* spp. (30%), *Alternaria* spp. (15%), and *Cladosporium* spp. (30%). Notably, the imported cucumber variety Björn F1 exhibited considerable susceptibility to multiple pathogens affecting both fruits and other plant organs. Furthermore, synergistic infections – where several pathogenic species simultaneously attacked a single plant – were observed, highlighting the need for integrated and targeted protective measures

Keywords: hybrid varieties of cucumber; fungal diseases of cucumber; identification of pathogens; morphological features; pathogenicity of pathogens

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Introduction

Cucumber remains one of the most popular crops grown in greenhouses in Kyrgyzstan. The State Register of Plant Varieties and Hybrids Approved for Use in the Kyrgyz Republic for 2024-2025 includes 121 cucumber varieties (Ministry of Agriculture, 2023). Recently, heterotic hybrids have become widespread, characterised by high yields and resistance to adverse conditions and diseases. Cucumbers are more susceptible to various diseases when grown in a humid greenhouse due to limited air circulation. They can be affected by a variety of fungal, bacterial and viral diseases that can destroy the entire crop if not treated and protected in a timely manner. Common diseases of cucumbers include powdery mildew, verticillium and fusarium wilt, root and basal rot, white rot and grey rot (which is common in greenhouse conditions).

Researchers around the world have identified the most common pathogens affecting cucumbers. Phytopathological monitoring conducted in the Jos Plateau ecological zone in Nigeria revealed the prevalence of ten phytopathogens from seven genera of fungi on cucumber crops, namely: *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Alternaria* spp., *Cladosporium* spp., *Colletotrichum* spp., *Fusarium solani*, *Fusarium oxysporum*, *Mycrosporium* spp. and *Penicillium* spp., with only *Colletotrichum* spp. proving to be pathogenic to cucumber seedlings. The highest and lowest prevalence was observed in *A. niger* and *Cladosporium* spp. – 17.1% and 4.9%, respectively (Shutt *et al.*, 2021). Field surveys were conducted in three areas of the local district of Keffi: Jigwada, Sharmaki and Yarkade, and samples of infected cucumber leaves were collected and analysed. The study revealed the presence of three major fungal diseases: downy mildew, anthracnose and leaf spot. Microscopic identification of fungal isolates from infected leaves identified ten different species of fungi, among which *Aspergillus niger* was the most common, accounting for 17.1% (Umar *et al.*, 2024).

In a study by C. Cheng *et al.* (2023), the roots, stems, and leaves of cucumber plants and their rhizosphere soil were collected twice separately from the field and greenhouse to isolate endophytic and rhizosphere soil fungi. Endophytic fungi and rhizosphere soil fungi were tested as biological control agents against phytopathogens or for their potential to stimulate cucumber growth. Three fungal pathogens (*Aspergillus flavus*, *Rhizopus stolonifer*, and *Aspergillus brasiliensis*) causing cucumber fruit rot were identified in the markets of Jimeta and Yola, north of Adamawa State (Nigeria). *Rhizopus stolonifera* had the highest (25-48%) frequency of occurrence, and *Aspergillus flavus* had the lowest (22-38%) (Jimeta *et al.*, 2022). Black spot on fruit is usually associated with the production of mycotoxins by toxigenic species of the genus *Alternaria*. To study this relationship, A. Saleem *et al.* (2022) obtained 20 *Alternaria* isolates from infected tomato fruits using a bait method. The isolates were identified to species level by morphological

analysis, the results of which were confirmed by sequencing the internal transcribed spacer (ITS) gene. The following species were identified: *A. alternata*, *A. brassicicola*, *A. citri*, *A. radicina*, and *A. tenuissima*.

The efficacy of *Trichoderma asperellum* strain T34 as a commercial biological product and potassium phosphate (KPHI) on *Pseudoperonospora cubensis*, the causative agent of downy mildew in cucumbers, was evaluated. The results of A. Abdelfatah *et al.* (2025) proved that T34 and KPHI can be environmentally safe alternatives to chemical fungicides for controlling downy mildew in cucumbers and other cucurbit crops. Vanillic acid (VA) from root exudates is commonly referred to as cucumber autotoxin, which affects the diversity and abundance of the soil microbial community. qPCR analysis showed that VA (0.05-0.2 mol/g soil) had a stimulating effect on the abundance of *Trichoderma* spp. and stimulated the abundance of *Fusarium* spp. at low concentrations (0.02-0.05 mol/g soil), but inhibited it at high concentrations (0.1-0.2 mol/g soil) (Chen *et al.*, 2018). *Aspergillus fumigatus*, *Fusarium* sp., *Geotrichum candidum* and yeast fungi were isolated and identified from infected cucumber fruits taken from the Gada-biu market (Nigeria). *Geotrichum candidum* had the highest (50%) occurrence of fungal isolates from all locations. All fungal isolates were pathogenic to cucumber fruits, with *Fusarium* being the most harmful, followed by yeast and *Geotrichum candidum*, and *Aspergillus fumigatus* being the least harmful (Ishaya *et al.*, 2019).

Several pathogens can infect a single host either simultaneously (co-infection) or sequentially over time (multi-infection) (Pandey *et al.*, 2025). Despite this, the effect of the order and timing of infection on disease severity, especially in fatal diseases, remains poorly understood. Pathogens are known to interact, influencing each other's pathogenicity through antagonistic or synergistic effects. Interactions between fungi and oomycetes are particularly common. For example, K. Foster *et al.* (2017) documented widespread synergistic associations between *Rhizoctonia* and *Pythium* spp.; *Pythium* and *Fusarium* spp.; *Pythium* spp. and *Aphanomyces trifolii*; and *Phytophthora clandestina* and *A. trifolii* on forage legumes in southern Australia. However, many such interactions probably remain unrecognised.

In Kyrgyzstan, the response of imported cucumber hybrids to the effects of phytopathogenic fungi and bacteria has not been sufficiently studied. The microclimate of protected soil, characterised by high humidity and limited air circulation, contributes to the intensive development and spread of pathogenic microflora. An effective system for protecting greenhouse crops requires reliable identification of disease pathogens. However, comprehensive phytopathological studies of cucumber diseases in open and protected ground conditions in the republic are practically absent, which does not allow characterising the morphobiological features and virulence of local pathogen isolates. The

aim of this study was to isolate and identify the species of fungal pathogens that mainly affect the fruits of the Björn F1 hybrid cucumber in greenhouse production, as well as to evaluate their pathogenic properties in relation to the host plant.

Materials and Methods

Observation site, disease symptoms and sample selection. On 1 August 2024, Björn F1 cucumbers were planted in the nursery of the Kyrgyz National Agrarian University named after K.I. Skryabin (KNAU). Tomatoes were the predecessors and were planted before the cucumbers. The total area of the greenhouse allocated for cucumbers was 20 m². The soil was enriched with manure (448.5 kg/m²) and mineral fertilisers (N₁₀₀, P₁₀₀, K₁₀₀) (448.5 kg/m²). The average temperature in the greenhouse was 16.6°C, and the air humidity was 89.3%. At the end of October – beginning of November, whitefly infestation was observed at a level of 10-15%,

accompanied by the appearance of bright symptoms of fungal damage on cucumber fruits. The assessment of disease symptoms on cucumbers in greenhouses and the percentage of disease incidence was carried out using random sampling, with at least 20 plants in the greenhouse being examined. The incidence was determined as the percentage of the total number of affected plants to the total number of plants examined. For phytopathological analysis, affected cucumber fruits with the following symptoms were selected: underdeveloped, blackened, covered with white felt-like fungal mycelium; shrivelled fruits with softened tissue and the onset of rot from the top; cucumbers of sufficient size, but with noticeable softening of the fruit tissue, starting with rotting from the top and covered with a felt-like coating (Fig. 1). The samples were delivered to the mycological laboratory of the Plant Protection Centre of the KNAU for the isolation and identification of pathogens.

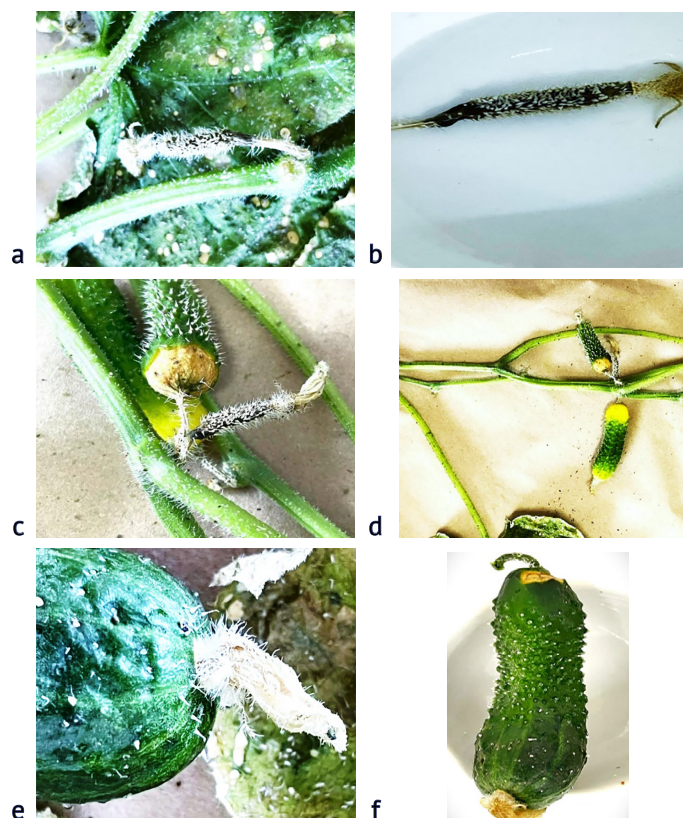


Figure 1. Samples of cucumber fruits with visible symptoms of disease taken for analysis

Note: symptoms on plants: a, b – underdeveloped blackened cucumber fruit covered with white felt-like coating and fungal mycelium; c, d – underdeveloped cucumber fruit: the fruit tissue begins to soften, it starts to rot from the top, the fruit looks shrivelled; e, f – larger cucumber fruit with softened tissue, it also starts to rot from the top and becomes covered with a felt-like coating
Source: authors' photos

The experiment was conducted in accordance with the ethical principles set out in the Convention on Biological Diversity (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (1973).

Isolation of pure cultures of the pathogen from samples. Cucumber fruits with symptoms of the disease

were thoroughly washed with tap water and cut into 5-10 mm pieces. The pieces were immersed in a 2% disinfectant solution (sodium hypochlorite). Then all samples were washed several times with distilled water, rinsed with sterile water and dried on sterile filter paper until completely dry. After drying, the samples were

transferred under sterile conditions to Petri dishes with nutrient agar. The dishes were incubated for seven days at 20°C and 97% relative humidity. After incubation, the primary colonies were separated based on the similarity of their cultural characteristics. These colonies were transferred to potato dextrose agar (PDA) and incubated for 48 hours at 20°C. Pure cultures were obtained by single spore isolation and cultivated on PDA and nutrient-deficient synthetic agar (SNA). For cultural studies, colonies were grown on PDA and SNA for 14 days at 25°C in the dark.

Morphological characterisation of pathogens. Pure cultures of fungal isolates were prepared for preservation and identification. Morphological characterisation of pathogens was performed using various nutrient media. Potato dextrose agar was used to evaluate the colour, texture and growth rate of colonies. SNA medium was used to study the formation and types of macroconidia, microconidia and conidiogenous cells. To identify the isolated fungi, standard phytopathological methods were used, with particular attention paid to morphological characteristics such as colony appearance, pigment formation, conidiophores, spores, and other morphological structures. Mycelial growth and the morphology of dried spores were studied in detail under a microscope. The fungal hyphae were stained with lactophenol and blue solution and observed under a MEIJI Advanced Compound Microscope Model ML5500 and a MEIJI Zoom Stereo Microscope Model EMZ-5TR-MA502-PBH (Japan), and microphotographs were obtained using a MOTIC 2.0 Mega Pixel Digital Microscope Camera with Images 2000 Software Model MOTICAM 2000. The number of septa, as well as the length and width of 30 conidia spores per isolate, were measured using an eyepiece micrometer under a light microscope. Identifiers (Williams-Woodward, 2001; Watanabe, 2010) were used to determine the species of fungi.

In vitro and in vivo pathogenicity tests. Pathogenicity was assessed both *in vitro* and *in vivo*. For the *in vitro* assessment, firm green apple varieties, namely

Simirenko and Golden Delicious, were selected to study the pathogenicity of fungal isolates obtained from infected cucumber fruits. Apples of approximately the same size and health status were thoroughly washed and then disinfected in a 2% sodium hypochlorite solution for 3 minutes. After disinfection, they were rinsed with sterile distilled water, dried, and inoculated with a suspension of 7-day-old pathogen cultures. Using a sterile 1.0 ml syringe needle, 1 ml of the suspension (1×10^6 conidia/ml) was injected to a depth of up to 1 cm into the skin of the fruit. Control apples received 1 ml of sterile water. All apples were then incubated at a constant temperature under sterile conditions for up to 20 days.

The pathogenicity of the isolates was evaluated *in vivo* on two-week-old tomato seedlings. Seedlings with intact root systems were immersed for two hours in a suspension (1×10^6 conidia/ml) of 7-day-old cultures of the pathogen isolated from diseased cucumbers. Control seedlings were treated with plain water. After immersion, all seedlings were planted in soil and kept at a constant temperature of 22-23°C with a 16-hour photoperiod. Sterile distilled water served as a negative control. To assess the aggressiveness of the isolates, symptoms were recorded weekly for four weeks after inoculation. The total duration of observation of the tested plants was 3 months.

Results

Cultural and morphological characteristics of *Cladosporium* pathogens isolated from cucumber fruits

Colonies grown on potato dextrose agar, which is a common medium for fungi (Fig. 2a), and synthetic agar with low nutrient content (Fig. 2b) grew slowly. The colour of the colonies varied mainly from olive brown to blackish brown, with rare shades from greyish olive to dull green. They had a velvety or flaky (soft, woolly) texture. The aerial mycelium, or threads growing above the surface, were rarely abundant, and the colonies did not produce any noticeable exudate (liquid secretion).

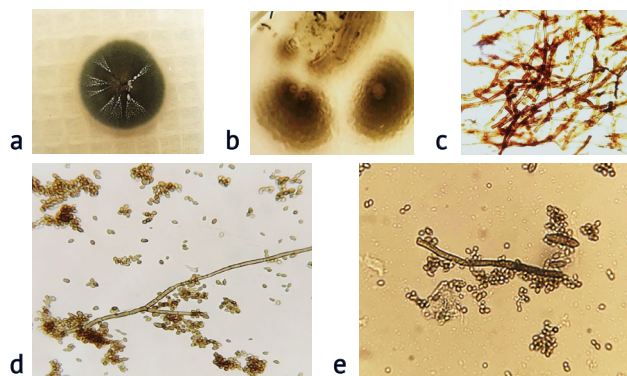


Figure 2. Morphological features of *Cladosporium cucumerinum* isolates

Note: a – colonies cultivated on potato dextrose agar (PDA); b – colonies developed on synthetic nutrient agar (SNA); c – microscopic observation of conidiophores; d – conidia formed from conidiogenous cells and apical regions; e – ramoconidia visualised under a microscope, $\times 400$

Source: authors' photos

To identify and determine the taxonomic affiliation of isolates 4(1) and 4(2), presumably belonging to the genus *Cladosporium*, their morphological structure was analysed based on the criteria established by B. Thomma *et al.* (2005) and K. Bensch *et al.* (2010). The results obtained were compared with published descriptions and photographs available on Microbe Notes (n.d.). The analysis revealed a number of distinctive features of *Cladosporium* isolates, including the presence of ramoconidia – spores that develop from segments of the conidiophore or its branches. These fungi also formed small conidia at the ends of their chains. Ramoconidia were 20–41 µm and 3.1–3.3 µm in size and formed long, loose chains, often with dichotomous branching. The mycelium of the colony consisted mainly of substrate and ranged in colour from almost colourless to brownish. Conidiophores were usually erect, septate, either unbranched or with one or two branches, often with dark pigmentation. Sometimes, micronematid-like conidiophores were observed, which were paler, unbranched, and 9 to 150 µm long. The apical unbranched part of these conidiophores contained up to 10 conidia. The terminal conidia varied from obovate to almost spherical, measuring 3–6 × 2–2.5 µm. In contrast, the middle conidia were lemon-shaped,

ellipsoidal-ovoid, sometimes almost cylindrical, 5–12 µm and 2.5–3 µm in size, without septa. The colour of the conidia varied from light brown to light olive brown, and the surface was smooth (Fig. 2). Based on these observations, isolates from affected cucumber fruits with pronounced symptoms of (Fig. 1a, 1b) were identified as *Cladosporium cucumerinum* (Ellis and Arthur). According to previous reports, this pathogen mainly affects fruits, less often leaves, causing characteristic spots on the fruits (Kwon *et al.*, 2000). This disease is known as olive (brown) spot of cucumbers.

Cultural and morphological characteristics of *Botrytis*, a pathogenic fungus isolated from cucumber fruit

Isolates 2.1, 2(2)-1 and 2(2)-2, presumably fungi of the genus *Botrytis*, showed distinctive cultural and morphological characteristics. When grown on nutrient media, the mycelium of these isolates proliferated rapidly, forming pronounced radial patterns and generating sclerotia. When cultivated on KDA, they formed fluffy, raised colonies characterised by grey-white airy mycelium with white edges and a green centre (Fig. 3a). Conidia began to appear on the mycelium within seven days of incubation, and black sclerotia formed after 14 days (Fig. 3b).

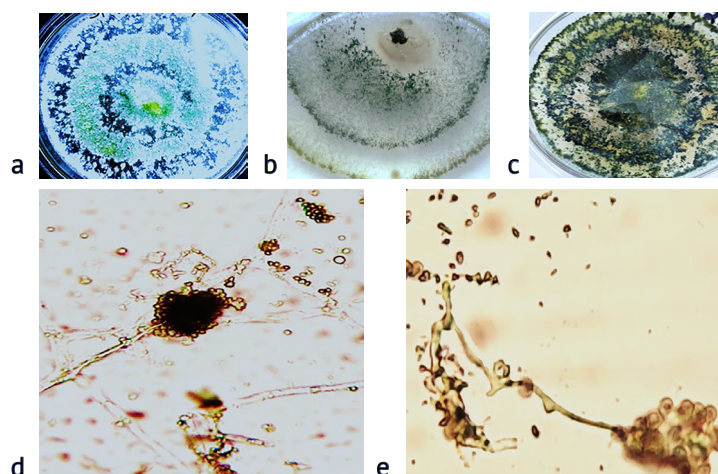


Figure 3. Morphological features of *Cladosporium cucumerinum* isolates

Note: a – colonies of isolates on KDA; b – colonies on SNA medium; c – colonies on day 7, showing the development of black sclerotia; d – transparent, straight conidiophores with apical phialides bearing round conidia; e – slightly pear-shaped, smooth, hyaline spores at the swollen ends of conidiophore branches, ×400

Source: authors' photos

The conidiophores were vertical, thin-walled and branched at the top. Their colour varied from greyish to light brown, and they were translucent, with a small number of transverse septa. At the swollen ends of the conidiophores, short sterigmata produced numerous single-celled spores that were slightly pear-shaped or spherical, smooth and transparent. Depending on the species and substrate, sclerotia ranged in size from 1 to 20 mm and could be round, ovoid or elliptical in shape. According to published descriptions and photographs available on Microbe Notes (n.d.), these isolates were identified as *Botrytis cinerea*, a member

of the Sclerotiniaceae family, a highly aggressive phytopathogen that causes grey mould. Affected plants developed darkened fruits, leaves, petals, and succulent stems, which then became soft, dried out, and eventually died. The succulent tissues of most plants are particularly vulnerable to grey mould. In addition, high humidity can lead to the rapid spread of *Botrytis cinerea*, causing buds, flowers, leaves and fruits to rot (Tanović *et al.*, 2014). Thus, the combination of morphological characteristics identified made it possible to reliably identify these isolates as representatives of the species *Botrytis cinerea*.

Cultural and morphological characteristics of *Fusarium* species isolated from cucumber fruits

The cultural and morphological characteristics of isolates 3.2, 3(2)-1 and 3(2)-2, presumably belonging to the genus *Fusarium*, were studied in detail. On SNA

medium, these isolates formed airy, silvery-white, fluffy colonies (Fig. 4a). When grown on KDA, they formed low, filamentous, cobweb-like, cotton-white colonies. As the mycelium matured, the colour of the colonies gradually changed to shades of pink and red (Fig. 4b).

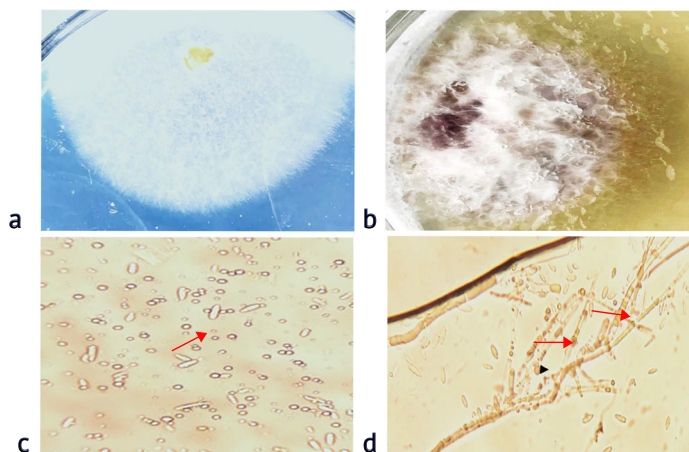


Figure 4. Morphological features of *Fusarium oxysporum* isolates

Note: a – colonies of isolates on KDA medium; b – colonies on SNA medium; c – microconidia; d – macroconidia, $\times 400$

Source: authors' photos

During further development, the fungus formed two different types of conidia. Macroconidia were spindle- or sickle-shaped with a clearly defined stalk or papilla, forming in aerial mycelium, sporodochia or pyconota. These macroconidia had a mostly uniform diameter, thin walls, a tapering base, and contained three to five septa measuring $23.5-51 \times 3.0-5 \mu\text{m}$ (Fig. 4d). The second type, microconidia, developed inside the mycelium and was abundant during the reproductive phases (Fig. 4c). These microconidia were elongated, unicellular, and colourless. In addition, the isolates produced chlamydospores, which were unicellular or multicellular, unstained, and thick-walled. These morphological features collectively closely resembled

those of *Fusarium oxysporum* f. sp. *cucumerinum*, a common pathogen in cucumber-growing areas (Din *et al.*, 2020). *Fusarium oxysporum* f. sp. *cucumerinum* is a specialised pathogen that causes fusarium wilt (tracheomycosis) in cucumbers. When it enters the host plant, it blocks the xylem vessels and secretes phytochemicals, then spreads through the vascular system, leading to systemic physiological disorders.

Cultural and morphological characteristics of *Alternaria* species pathogenic to cucumbers, isolated from fruits

Isolates 5(1) and 5(2) formed dense, felt-like colonies on PDA, ranging in colour from black to dark grey with clearly defined rounded outlines (Fig. 5a, 5b).

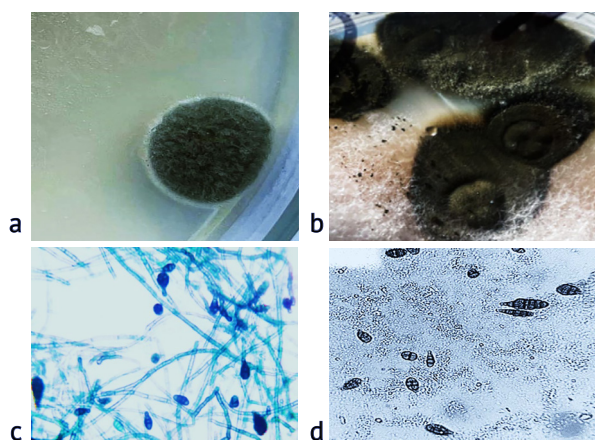


Figure 5. Morphological features of *Alternaria* spp. isolates

Note: a – colonies of isolates on PDA (young culture); b – colonies on PDA (mature culture); c – conidiophores with conidia; d – conidia, $\times 400$

Source: authors' photos

The surface of these colonies was uniformly velvety, with clearly defined smooth edges. Species of the genus *Alternaria* are characterised by large, multicellular, dark-pigmented conidia with transverse and longitudinal septa (Fig. 5c, 5d). These conidia are usually oblong or pear-shaped, but may also be ovoid or ellipsoidal, often with a short conical or cylindrical tip. Their walls are pale brown and may be smooth, finely warty or coarsely rough. Traditionally, these fungi are divided into two groups based on morphological and molecular phylogenetic characteristics: group 1, which has finely warty walls, and group 2, which has coarsely warty or rough walls.

Pathogenicity of isolated fungal strains *in vitro*

Fungi can act as pathogens, using various strategies to colonise and penetrate plant tissues, ultimately leading to disease. Some fungi are necrotrophs, meaning they kill their host plants and feed on dead tissue. Biotrophic fungi, on the other hand, colonise living tissues

without immediately killing their hosts. To effectively penetrate plant organs, fungi develop specialised infectious structures and produce a range of hydrolytic enzymes and toxins that destroy plant tissues (Doehlemann *et al.*, 2017). It should be noted that necrotrophic fungal pathogens are similar to biotrophic ones. Both types secrete small molecules known as effectors, which interact with the host at the genetic level, initiating pathological processes (Oliver & Solomon, 2010). A striking example of a significant necrotrophic phytopathogen is *Botrytis cinerea*, which has a wide range of hosts. In addition, species of the genus *Cladosporium* are non-obligate biotrophic fungi, causing tomato leaf blight, in particular *Cladosporium fulvum* (also known as *P. fulva*) (Thomma *et al.*, 2005). It is important to note that fungi of the genus *Cladosporium* do not cause necrotic rot on apple fruit (Fig. 6i). This is probably due to their biotrophic nature: instead of secreting enzymes that damage plant tissue, they use other strategies to infect plants.

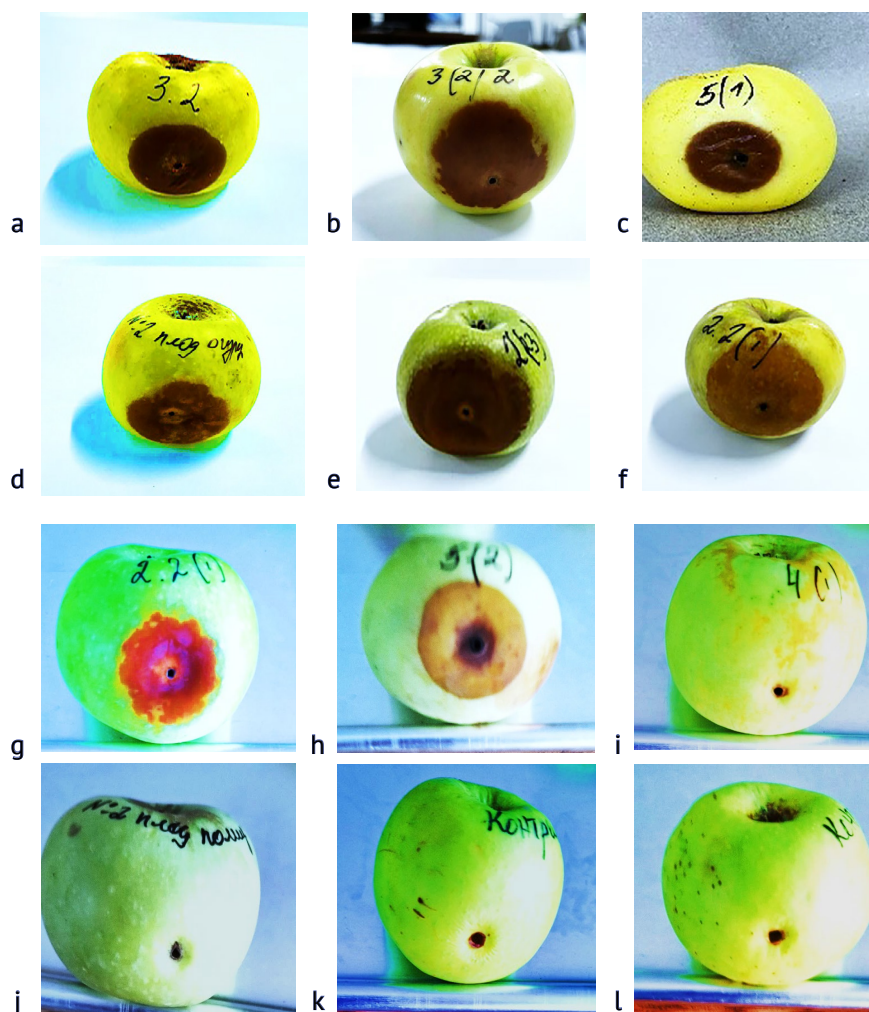


Figure 6. The appearance of necrotic spots, or rot, on apple fruit after artificial inoculation with pathogenic fungal cultures

Note: panels a and b show isolates of the genus *Fusarium*; panels c and h show isolates of the genus *Alternaria*; panels d, e, f, and g show isolates of *Botrytis cinerea*; panel i shows an isolate of the genus *Cladosporium*; panels j, k, and l show control fruits

Source: authors' photos

Various pathotypes of *Alternaria alternata* produce at least 12 different host-specific toxins (HSTs) that enable them to cause disease in various dicotyledonous plants (Thomma, 2003). In experiments involving artificial inoculation with these pathogens in the present study, typical symptoms were observed, and repeated isolation of the fungi confirmed Koch's postulates. The study of pathogenicity on apple fruit revealed clear symptoms characterised by superficial watery lesions. The affected tissue took on a brownish tint. Virtually all isolated fungal strains produced positive symptoms on green apple varieties with firm fruit (Fig. 6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h). After 7 days, necrotic lesions formed on the fruits surrounding the inoculation site, while no necrosis was observed at the injection site of the control fruits (Fig. 6j, 6k, 6l). This suggests that the isolated fungi are necrotrophs that produce hydrolytic enzymes,

including pectinases, which break down pectin in apple tissues (Asoufi *et al.*, 2007). The results confirm the pathogenic activity of the isolated strains and their ability to enzymatically destroy plant tissues *in vitro*.

Pathogenicity of the isolated fungal strains *in vivo*

Pathogenicity was assessed *in vivo* on two-week-old tomato seedlings. During the three-month observation period, it was evident that all tested pathogens had a significant effect on tomato plant growth. Tomato plants inoculated with cultures from the genera *Fusarium*, *Alternaria*, *Botrytis*, and *Cladosporium* showed similar disease symptoms, including yellowing of leaves and wilting. In contrast, the control plants remained healthy after a four-week incubation period, showing no signs of yellowing, chlorosis or wilting. Symptoms of yellowing began on older leaves, which then progressed to chlorosis and wilting (Fig. 7).

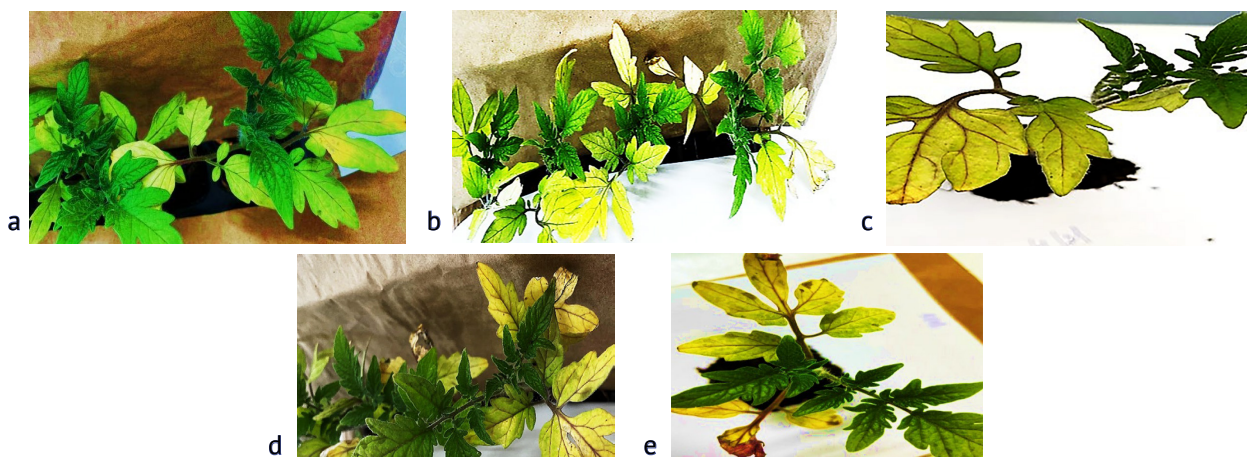


Figure 7. Symptoms of disease on tomato seedlings 20 days after inoculation

Note: a – control plants without symptoms, b – yellowing and wilting of leaves infected with *Cladosporium* isolates; c – *Alternaria* isolates; d – *Botrytis* isolates; e – *Fusarium* isolates

Source: authors' photos

The results showed that *Cladosporium*, *Fusarium* and *Alternaria* isolates were more aggressive than *Botrytis* pathogens. To confirm these results, infected

tomato leaves and stems were re-isolated, resulting in the formation of pathogen colonies confirming Koch's postulates (Fig. 8).

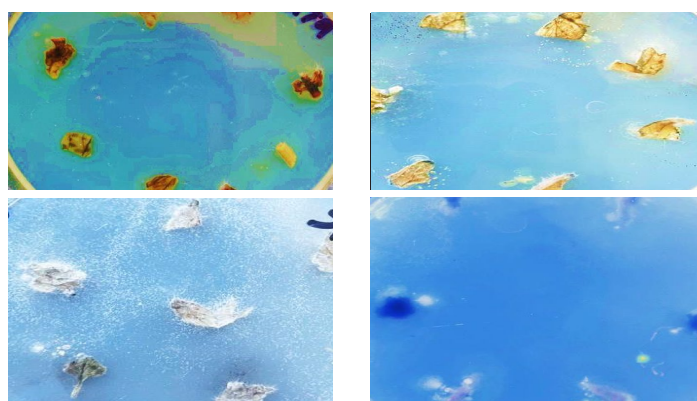


Figure 8. Primary pathogen colonies grown from leaves and stems of plants with visible symptoms

Source: authors' photo

Re-isolation of pathogens from affected tomato plant organs demonstrated the formation of primary fungal colonies, which were subsequently transferred to KDA medium to obtain pure cultures with morphological characteristics specific to each species. The successful isolation of the original pathogens from infected tissues finally confirmed their aetiological role in the development of the disease. Detailed results of this stage of the study will be presented in subsequent publications.

Discussion

The present study showed that cucumbers grown in greenhouses, especially in October and November, are susceptible to mixed infections caused by several pathogens simultaneously. Assessment of disease symptoms in the field allowed for collecting fruits with signs of wilting, rotting, and other visible symptoms. For accurate identification of the pathogen, classical phytopathological methods and microscopic morphological analysis were performed, as described in specialised sources on fungal identification. The results showed that several species of killer pathogens can parasitise the fruit at once, their action is aimed at the effective destruction of host cells, and as necrophages, they feed on dead cells. During the study, necrophages such as species of the genera *Fusarium*, *Botrytis* and *Alternaria* were identified. Their pathogenicity was proven in *in vitro* and *in vivo* tests. In *in vitro* tests, they showed the ability to produce hydrolytic enzymes that decompose the hard tissue of apple trees, consisting of pectin compounds and other mono- and disaccharides. An interesting fact was noted under *in vivo* conditions, where two-week-old tomato seedlings were used as test hosts, since these same pathogens can use tomato plants as hosts. It is known that tomatoes and cucumbers have common pathogens, especially since tomatoes were the predecessors of cucumbers in the greenhouse. Ten days after artificial infection by soaking the roots of the seedlings in a suspension of pathogenic cultures, symptoms began to develop on the lower, earlier-appearing leaves, including yellowing and wilting. In contrast, the control plants remained healthy after a four-week incubation period, showing no signs of yellowing, chlorosis or wilting. The symptoms of yellowing progressed to chlorosis and wilting.

It should be noted that all pathogens had similar symptoms, which manifested themselves primarily on the leaves; during the experiment, no damage to other organs, such as the root system, was observed. At the same time, all diseased plants lagged behind in growth and did not enter other phases of vegetation, such as flowering and fruiting. The wilted leaves died, and no new leaves appeared, or their development was slow. According to many authors, pathogens secrete phytochemicals that cause the death of host plant cells (Friesen et al., 2008). For example, *B. cinerea* secretes botrydial during the infection of plants, which causes leaf necrosis,

chlorosis and tissue wilting (Staats et al., 2005). In addition, the pathogen *Fusarium oxysporum* secretes SIX effector proteins (proteins secreted in xylem) into the xylem, promoting infection (Din et al., 2020). The data obtained in this study also indicate the widespread distribution and aggressiveness of the pathogen *Fusarium oxysporum* f. sp. *Cucurbitacearum* (the causative agent of *Fusarium* wilt), which is confirmed by other authors (Shen et al., 2008; Din et al., 2020). At the same time, a study by M. Pikovskyi et al. (2023) showed that the optimal temperature for the vegetative growth of *Fusarium oxysporum* f. sp. *cucumerinum* is 30°C, and the most intense spore formation of the pathogen occurs at a temperature of 25°C.

The prevalence of another pathogen, *Alternaria alternata*, in the study was 15%, which is consistent with the data of other authors who note this species as a potential pathogen of cucumbers in both open and closed soils (Thomma et al., 2005; Hubballi et al., 2011). Among the species found in this study, *Botrytis cinerea* (the causative agent of grey mould) was represented by several strains, with a prevalence of up to 30% among the species found. The frequency of detection of this species in cucumber crops has also been noted by other authors, such as B. Tanović et al. (2014). It should be noted that cucumber pathogens are represented by different species on different continents and in different parts of the world. For example, in African countries, the species *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus* dominate (Shutt et al., 2021; Umar et al., 2024). Meanwhile, in Central Asia, *Cladosporium* spp., *Fusarium oxysporum*, and *Botrytis cinerea* are the main ones. In other Asian countries, like China, *Fusarium oxysporum* is the main one on cucumber crops (Ye et al., 2004; Shen et al., 2008). In Eastern European countries, particularly Ukraine, *Pseudoperonospora cubensis*, the causative agent of downy mildew, is widespread (Bondarenko & Stankevych, 2021).

Thus, in autumn, in greenhouse conditions, with humidity of about 90% and a temperature of 16.6°C, favourable conditions are created for the development of several types of pathogens simultaneously. The high infectious background is also due to the predecessor plant, tomato, which is affected by the same pathogens as cucumber. The imported hybrid Björn F1 variety showed high sensitivity to fungal pathogens such as *Fusarium* spp., *Botrytis* spp., *Alternaria* spp., and *Cladosporium* spp., although the producers and creators of this variety emphasised its resistance to *Cladosporium*.

Conclusions

Under protected ground conditions, the microclimatic characteristics of greenhouses create a favourable environment for the simultaneous development of a complex of phytopathogenic microorganisms capable of rapidly colonising various organs of the host plant. The studies conducted have convincingly demonstrated

that the visually recorded symptoms of cucumber fruit damage are caused by a polyetiological infection, which indicates the formation of associative pathocomplexes during the development of the disease. Cultural and morphological analysis revealed the presence of reproductive structures and mycelium of several species of fungal pathogens within a single affected fruit, indicating synergistic interaction between pathogens during the colonisation of plant tissues.

Phytopathological studies have established the high susceptibility of Björn F1 hybrid cucumber fruits to pathogenic fungi of the genera *Fusarium*, *Botrytis*, *Cladosporium*, and *Alternaria*. The data obtained emphasise the need for strict compliance with agrotechnical requirements, including scientifically based selection of predecessors, timely preventive measures and maintenance of optimal microclimate parameters when cultivating hybrid cucumber varieties in protected ground. When developing integrated plant protection systems,

it is critically important to consider the possibility of polyinfection, when several types of pathogens causing diseases of different aetiologies simultaneously parasitise one plant. Prospects for further research include molecular genetic identification of isolated strains, studying the mechanisms of pathogen interaction in mixed infections, and developing effective biological and chemical control measures for identified pathogens, taking into account their synergistic effects in the protected soil conditions of Kyrgyzstan.

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Conflict of Interest

None.

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Күнөскана шарттарында ар түрдүү козу карын түрлөрүн чакырган бадыраң мөмөлөрүнүн аралаш инфекциялары

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Аннотация. Бадыраң Кыргызстанда күнөсканаларда өстүрүлгөн эң популярдуу өсүмдүктөрдүн бири. Акыркы убакта партенокарпиялык гетерозис гибриддер күнөсканада өстүрүү үчүн кеңири колдонулууда. Бирок, гибридик сорттордун ар кандай козу карын же бактериялык илдеттерге туруктуулугу же сезгичтиги изилденбеген бойдон калууда, анткени парниктерде нымдуулук жана аба агымынын чектелгендигинен илдет козгогучтар үчүн жагымдуу шарттар түзүлөт. Бадыраңдын илдеттерин так аныктоо түшүмдү өз убагында коргоо үчүн зарыл. Кыргызстанда ачык жана жабык жерде өстүрүлгөн бадыраңдарга таасир этүүчү илдет козгогучтар алардын айырмаланган морфологиялык жана патогендик касиеттерин аныктоо адистештирилген фитопатологиялык ыкмалар менен изилдене элек. Бул изилдөөнүн максаты күнөсканада өстүрүлгөн Björn F1 бадыраңына таасир этүүчү козу карын козгогучтарын бөлүп алуу жана аныктоо, алардын кожоюн өсүмдүккө карата патогендүүлүгүн аныктоо болуп саналат. Изилдөө үчүн илдеттин белгилери жакшы көрүнгөн бадыраңдардын мөмөлөрү тандалып алынган. Илдет козгогучтарды аныктоодо фитопатология жана микробиология илимдеринин заманбап жана классикалык ыкмалары колдонулуп, жарык микроскопиясынын жардамы менен алардын культуралдык-морфологиялык мүнөздөмөлөрү изилденди. Изилдөөдө *Fusarium*, *Botrytis*, *Alternaria* жана *Cladosporium* урууларына таандык козу карын патогендери аныкталды. Алардын патогендүүлүгү *in vitro* жана *in vivo* шарттарында көрсөтүлдү. Табылган патогендердин штамдарынын таралыш пайызы: *Fusarium* spp. – 25 %, *Botrytis* spp. – 30 %, *Alternaria* spp. – 15 %, *Cladosporium* spp. – 30 %. Бул изилдөөнүн маанилүү жыйынтыгы болуп импорттолгон бадыраң Björn F1 сортунун бир эле мезгилде бир нече патогендерге сезгичтиги аныкталды. Бир өсүмдүккө бир нече патогендик түрлөрдүн синергетикалык чабуулу байкалды, мындай кубулуш коргоо чараларын тандоодо жана ишке ашырууда эске алынышы маанилүү.

Негизги сөздөр: бадыраңдын гибридик сорттору; бадыраңдын козу карын илдеттери; козгогучтарды аныктоо; морфологиялык өзгөчөлүктөрү; козгогучтардын патогендүүлүгү

Смешанные инфекции плодов огурцов, вызванные различными грибковыми возбудителями в условиях теплицы

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Аннотация. Огурец – одна из самых популярных культур, выращиваемых в защищенном грунте в Кыргызстане. Для выращивания в теплицах в последние годы широко используются партенокарпические гетерозисные гибриды. Однако устойчивость или восприимчивость гибридных сортов к различным грибным или бактериальным болезням остается неизученной, и это вызывает серьезные опасения, так как в теплицах создаются благоприятные для патогенов условия из-за влажности и ограниченности потока воздуха. Для своевременной защиты урожая нужна точная диагностика болезней огурцов. В Кыргызстане до сих пор не изучены возбудители болезней огурцов, выращенных в открытом и закрытом грунтах, с применением специальных фитопатологических методов для выявления отличительных морфофизиологических и патогенных свойств патогенов. Целью настоящего исследования были изоляция и идентификация возбудителей грибных болезней, проявленных в первую очередь на плодах огурцов сорта Бьерн F1, выращенных в тепличных условиях, выявление их патогенности в отношении растения-хозяина. Для анализа были отобраны больные плоды огурцов с симптомами болезней. Для выявления патогенов были использованы современные и классические методы фитопатологии, микробиологии, и для их идентификации были изучены культуральные и морфологические особенности с помощью световой микроскопии. В результате исследований были выявлены грибные болезни и идентифицированы их возбудители, относящиеся к родам *Fusarium*, *Botrytis*, *Alternaria* и *Cladosporium*. Была определена их патогенность в *in vitro* и *in vivo* условиях. Процент доминирования штаммов составил: *Fusarium* spp. – 25 %, *Botrytis* spp. – 30 %, *Alternaria* spp. – 15 %, *Cladosporium* spp. – 30 %. Важным результатом было установление восприимчивости импортированного сорта огурцов Бьерн F1 к нескольким патогенам, поражающим плоды и другие органы растений. Установлена синергическая атака сразу нескольких видов патогенов на одно растение, что важно учитывать при подборе и организации защитных мероприятий

Ключевые слова: гибридные сорта огурца; грибные болезни огурца; идентификация возбудителей болезней; морфологические особенности; патогенность возбудителей